AD-A149 351

UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010-5422

FINAL REPORT
PRELIMINARY ASSESSMENT OF RELATIVE TOXICITY AND MUTAGENICITY POTENTIAL OF 1-NITROSO-3,5-DINITRO-1,3,5,-TRIAZACYLOHEXANE (MONONITROSO-RDX), STUDY NO. 75-51-0345-85
OCTOBER 1982 - SEPTEMBER 1984

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genotoxicity	nitrosamine	
irritation	1-nitroso-3,5-dinitro-1,3,5-triazacyclohexane	
mononitroso-RDX	PDX	
munition	sensitization	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The munitions component, 1-nitroso-3,5-dinitro-1,3,5-triazacyclohexane (Mononitroso-RDX), was assessed for its relative irritant, skin sensitization, and mutagenicity potential. It showed no potential for eye or skin irritation in rabbits following a single application. It did not prove to be a skin sensitizer to guinea pigs. Mononitroso-RDX showed positive mutagenic activity in three of four *in vitro* bioassays. This finding, in addition to its implicating chemical structure, would earmark the chemical as a suspect mutagen and/or carcinogen. Further testing is recommended to establish human risk. Interim protection and

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20. - monitoring of operational personnel is also recommended until mutagenic/ toxicological risks are resolved.

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Mr. Snodgrass/slw/AUTOVON
584-3980

REPLY TO
ATTENTION OF

HSHB-OT/WP

20 DEC 1984

SUBJECT: Final Report, Preliminary Assessment of Relative Toxicity and Mutagenicity Potential of 1-Nitroso-3,5-dinitro-1,3,5-triazacylohexane (Mononitroso-RDX), Study No. 75-51-0345-85, October 1982 - September 1984

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Copies of subject report with Executive Summary are enclosed.

FOR THE COMMANDER:

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Environmental Health

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HQDA(DASG-PSP) (wo/encl)
Cdr, ARRADCEN [AMSMC-LCE-D(D)] (w/encl)
Cdr, HSC (HSCL-P) (w/encl)
Comdt, AHS (HSHA-IPM) (w/encl)
Cdr, AMCCOM [AMSMC-SG(R)] (w/encl)
Cdr, MEDDAC, Ft Monmouth (PVNTMED Svc) (w/encl)
Cdr, WRAMC (PVNTMED Svc) (w/encl)
Cdr, USAEHA-Rgn Div North (w/encl)

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REPLY TO
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EXECUTIVE SUMMARY
FINAL REPORT
PRELIMINARY ASSESSMENT OF RELATIVE TOXICITY AND MUTAGENICITY POTENTIAL OF 1-NITROSO-3,5-DINITRO-1,3,5,-TRIAZACYLOHEXANE (MONONITROSO-RDX), STUDY NO. 75-51-0345-85 OCTOBER 1982 - SEPTEMBER 1984

1. PURPOSE. Mononitroso-RDX is a munitions component which may present a potential for occupational exposure. As such, its relative irritant and skin sensitization potential in animals was assessed. A battery of short-term mutagenicity assays were also performed to identity potential carcinogenicity risks in man.

2. ESSENTIAL FINDINGS. Mononitroso-RDX showed no potential for eye or skin irritation in rabbits following a single application. It did not prove to be a skin sensitizer to guinea pigs. The subject chemical demonstrated positive mutagenic activity in three out of four in vitro assays but not in the in vivo dominant lethal bioassay. This finding, in addition to its implicating chemical structure (nitrosamine), would earmark Mononitroso-RDX as a suspect mutagen and/or carcinogen.

3. RECOMMENDATIONS. It is recommended that confirmation testing be performed in live animal systems to measure gene mutations, chromosome alterations, primary DNA damage, and tumor induction. More intensive toxicological evaluations are also recommended to establish the effects of anticipated human exposures and risk assessment. It is recommended that interim protection and health monitoring of occupationally exposed personnel be instituted until questions of mutagenic/toxicological risks are resolved. Appropriate warning labels should be posted.

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010-5422

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FINAL REPORT
PRELIMINARY ASSESSMENT OF RELATIVE TOXICITY AND MUTAGENICITY POTENTIAL OF 1-NITROSO-3,5-DINITRO-1,3,5,-TRIAZACYLOHEXANE (MONONITROSO-RDX), STUDY NO. 75-51-0345-85 OCTOBER 1982 - SEPTEMBER 1984

1. AUTHORITY.

a. Letter, DRDAR-LCE, ARRADCOM, 23 July 1981, subject: Toxicological Hazard of N-Nitroso, N'N" Dinitro Triazine.

b. Letter, DRSMC-LCE-D(D), ARRADCEN, 28 October 1983, subject: Toxicological Hazard of N-Nitroso-N'N"-Dinitro-Hexahydro-Triazine, with indorsement thereto.

2. REFERENCES.

a. Preliminary Assessment of Relative Toxicity of 1-Nitroso-3,5-dinitro-1,3,5-triazacyclohexane (Mononitroso-RDX), Study No. 75-51-0345-82, US Army Environmental Hygiene Agency Report, (July 1981 - April 1982).

b. Toxicology Division, Topical Hazard Evaluation Program Procedural Guide, US Army Environmental Hygiene Agency, January 1982.

3. PURPOSE. The purpose of this compendium is to summarize previously reported information (reference 2a) concerning the relative irritant and sensitization potential of Mononitroso-RDX in animals and the more recent results of a battery of in vitro and in vivo screening programs for assessing mutagenicity potential. The latter tests were indicated because the subject compound belongs to a structural class of chemicals having known mutagenic/carcinogenic activity. The collective information will aid in advising on possible toxicological risks associated with the handling and use of this compound.

4. SPONSOR. US Army Armament, Munitions and Chemical Command, DRSMC-LCE-D(D), Dover, NJ 07801-5001.

5. GENERAL.

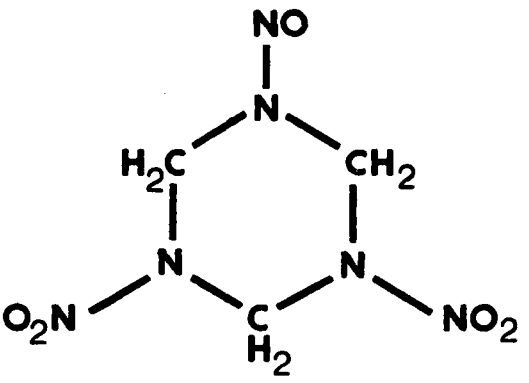
a. See Appendix A for the Bibliography.

b. See Appendix B for Analytical Quality Assurance information.

6. BACKGROUND. Mononitroso-RDX is a new munition component being tested by the US Army Research and Development Center (ARRADCEN). As such, a potential for occupational exposure exists among operational personnel.

7. MATERIALS.

a. Mononitroso-RDX is a light yellow powder with a molecular weight of 206. It is an insensitive explosive. It has an empirical formula of $C_3H_6N_6O_5$ and is identified by the following structure:



b. The compound was provided by Dr. Sury Iyer, LCWSL, ARRADCEN, Dover, NJ 07801-5001. Telephone (AUTOVON) 880-2525.

8. METHODS.

a. The methods used in the acute eye and skin irritation tests and the guinea pig sensitization study were previously reported (reference a).

b. The in vitro basic genetic toxicology screening assays were performed under contract number DAADO5-82-M-C279 by Litton Bionetics (LBI), Incorporated, 5516 Nicholson Lane, Kensington, MD 20895. These included:

(1) Ames Salmonella/Microsome Plate Test, LBI, Protocol No. 401.

(2) Mouse Lymphoma Forward Mutation Assay, LBI Protocol No. 431.

(3) Cytogenetic Assay, Chinese Hamster Ovary (CHO), LBI Protocol No. 437, Edition 7.

(4) Primary Rat Hepatocyte, Unscheduled DNA Synthesis Assay, LBI Protocol No. 447, Edition 5.

c. An in vivo assay, Dominant Lethal Effects of Mononitroso-RDX in mice, was performed under contract number DAAD 0582-M-C283 by Omni Research, Incorporated, 4800 Roland Avenue, Baltimore, MD 21210.

9. SUMMARY OF FINDINGS. A literature search on the subject compound revealed no CAS number or chemical or toxicological information. However, as a class, nitrosamines have been been vigorously investigated with respect to their genetic toxicity potential. A tabular presentation of animal toxicity data*† developed in this laboratory and genetic toxicity data submitted by the contractors follows:

TABLE. PRESENTATION OF DATA.

TEST	RESULTS	INTERPRETATION
SKIN IRRITATION STUDIES		
Rabbits		
Single 24-hour application to intact and abraded skin. Dry, technical grade chemical, 0.5 gm, applied to each of six rabbits. Occlusive covering.	Chemical did not cause any irritation of the intact or abraded skin.	Chemical exhibits no potential for acute skin irritation and should be considered a nonirritating skin material.
EYE IRRITATION STUDY		
Rabbits		
Single 24-hour application of 0.1 gm dry technical grade chemical to one eye of each of six rabbits. Three additional rabbits had the chemical washed out after a 20-second exposure.	Chemical did not cause irritation to the eyes of rabbits under either unwashed or washed conditions.	Irritation to human eyes is not expected from an accidental exposure.

* In conducting the studies described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," US Department of Health, Education and Welfare Publication No. (NIH) 78-23, revised 1978.
† The experiments reported herein were performed in animal facilities fully accredited by the American Asscciation for the Accreditation of Laboratory Animal Care.

TEST	RESULTS	INTERPRETATION
SENSITIZATION STUDIES		
Guinea Pigs		
Intradermal (ID) injections of 0.1 mL of a 0.1 percent solution (w/v) of the tested chemical in a mixture containing 1 volume of propylene glycol and 9 volumes of saline. Skin sensitizer, dinitrochlorobenzene (DNCB) was used in a mixture containing 1 volume of propylene glycol and 29 volumes of saline.		
Ten guinea pigs each received 10 sensitizing injections of mononitroso-RDX over a 3-week period. After 2 weeks rest, they were challenged with a single ID injection of the test chemical.	A challenge dose of the test chemical did not produce a sensitization reaction in guinea pigs.	Mononitroso-RDX is not expected to produce a sensitization reaction in man.
Ten positive control guinea pigs were sensitized over a 3-week period with DNCB and challenged 2 weeks later.	A challenge dose of DNCB produced a marked sensitization reaction in 10 out of 10 guinea pigs.	DNCB produced a marked reaction, indicating that guinea pigs respond to strong sensitizing agents.

ASSAY	CLASS OF EVENT DETECTED	CONCLUSIONS
In Vitro GENOTOXICITY ASSAYS		
AMES TEST Salmonella indicator organisms (5), tested directly and in presence of liver microsomal enzyme preparations from Arochlor® induced rats.	Specific Locus Gene Mutation	Mononitroso-RDX did not exhibit mutagenic activity.
MOUSE LYMPHOMA FORWARD MUTATION ASSAY. Mammalian cells, mutations induced at the thymidine kinase (TK) locus, with and without S9 metabolic activation.	Specific Locus Gene Mutation	Mononitroso-RDX induced significant increases in mutant frequency at the TK locus in mouse lymphoma cells. Dose-dependent increases in mutant frequency were induced with and without metabolic activation. The test material is, therefore, considered active.
CYTOGENIC ANALYSIS IN CHO CELLS. Chinese Hamster Ovary cells, with and without S9 metabolic activation.	Chromosome Aberrations (breaks or number changes)	The aberration test with the S9 activation system was clearly positive for Mononitroso-RDX. All kinds of chromosome aberrations, including the complex chromatid interchanges, were observed. No significant increase in aberrations was found in the test without activation.

® Arochlor is a registered trademark of the Monsanto Company, St. Louis, Missouri. Use of trademarked names does not imply endorsement by the US Army, but is intended only to assist in identification of a specific product.

ASSAY	CLASS OF EVENT DETECTED	CONCLUSIONS
In Vitro GENOTOXICITY ASSAYS		
PRIMARY RAT HEPATOCYTE, UNSCHEDULED DNA SYNTHESIS (UDS). Mammalian cell culture; unspecified DNA damage; repair indicated by ^{3}H-thymidine uptake.	Stimulation of DNA Repair Systems	Mononitroso-RDX induced significant increases in nuclear labeling of primary rat hepatocytes (repair) and is therefore considered active in the UDS Assay.
In Vivo GENOTOXICITY ASSAY		
DOMINANT LETHAL EFFECTS IN MICE. Five oral doses to males (sub-acute). Each mated with two females per week x7. Females assessed for early and late fetal deaths, fertility index.	Chromosome breaks in male germ cells	The positive control Triethylenemelami (TEM) induced dominant lethal effects in weeks 1, 2, and 3. The test compound, Mononitroso-RDX, did not induce a dominant lethal effect in mice.

10. DISCUSSION.

a. The primary objective of short-term mutagen testing is to determine whether a chemical has the potential to cause heritable genetic alterations in man. The rationale being that genetic material and the heredity processes are similar in all living organisms thus having relevance to the question of human risk. Although it is accepted that while all mutagens are not carcinogens, nearly all carcinogens are mutagens. No single method for the assessment of mutagenic potential exists because mutations occur at several levels of chromosome organization. It is, therefore, important that a battery of genotoxicity tests be performed that measure distinctly definable genetic events, i.e., point mutations and chromosomal effects. Because a single short-term test may yield an occasional false positive or false negative result, the battery approach requires that no conclusion should be drawn or decision made without all the data being collectively considered. Also to be considered is the structure of the chemical. If it is a potential electrophilic reactant or structurally related to known carcinogens, the risk is obviously increased.

b. Mononitroso-RDX is considered a cyclic nitrosamine by virtue of its chemical structure (NNO attached to an aryl group).[1] As a class, the nitrosamines are considered potent carcinogens which, in vivo, act as alkylating agents.[2] This is not to say that the subject compound is indeed carcinogenic, but does place it within a class of chemicals which have collectively produced more known genotoxic compounds than any other single group.[3]

c. Of the five genotoxicity assays reported here, three indicate positive mutagenic activity following Mononitroso-RDX treatment. The positive tests represent three distinct genetic events: specific locus gene mutation, chromosome aberrations and unscheduled DNA synthesis (as measured by repair). Of these events, DNA repair is a specific response to DNA damage and cannot be attributed to cytotoxicity.[4] Damage in the hepatocyte repair test strongly indicates coavalent building to DNA, an established property of carcinogens and mutagens.[4] Thus, it (DNA repair assay) provides an endpoint of high specificity and biological significance. The remaining positive tests tend to support rather than extend the significance of these observations. Therefore, the tests are generally regarded as qualitative rather than quantitative predictors.[5]

d. The Ames test and the in vivo dominant lethal bioassay were both negative. The Ames test may or may not be significant in predicting the mutagenic potential of the nitrosamines. It has been reported that of the 50 or so potently carcinogenic nitroso compounds tested, that about one-third (16) were not Ames positive.[6] The absence of Mononitroso-RDX effects in the dominant lethal bioassay is of interest because clastogenic events (chromosome breaking) were observed in the chromosome aberration assay using chinese hamster ovary cells. Similar aberrations might be expected in the mouse germinal cell resulting in wastage of the progeny. The variance of the two assay systems, in vitro versus whole animal, suggests that the chemical may not reach the target cell DNA (mouse) in an active form.[7] Further, a significant portion of chromosomal mutations may be "screened out" in meiosis and, therefore, not appear in mature sperm.[7]

e. In summary, a battery of short-term genotoxicity tests can be used as a qualitative predictor of human response because of the high positive correlation between the carcinogenic and mutagenic activities of chemical carcinogens. The assays reported in this study represent a consensus state-of-the-art approach.[5] Short-term positive results can be regarded as support for a conclusion of potential human carcinogenicity. Mononitroso-RDX, by virtue of its chemical structure and demonstrated mutagenic activity, should be considered a suspect human carcinogen. Prechronic, limited in vivo bioassays are indicated to develop further evidence of the carcinogenic potential. Unlike the in vitro tests, these are not applied as a battery but rather are used selectively to confirm detection of a specific endpoint. In the case of Mononitroso-RDX, these end points should include in vivo confirmation of gene mutation, chromosome aberrations, and primary DNA damage. A relatively short-term bioassay (40 weeks or less) attempting to chemically induce tumors in rodents may also be indicated since most chemicals active in the short-term systems (i.e., Shimkin lung tumor induction test[8]) are also carcinogenic in long-term animal tests.[4] In addition, intensive toxicological evaluations, to include pharmacckinetics, are necessary to elucidate dose-response, minimum effective dose, elimination rate from the body, and target organ disposition. The above recommended tests represent the minimum requirement for confirming the short-term genotoxicity results, estimating the doses for continued carcinogenic testing, and providing additional data for a rational assessment of human risk.

11. RECOMMENDATIONS. Based on the demonstrated mutagenic activity of Mononitroso-RDX, the following recommendations are offered:

a. Perform additional confirmation testing in live animals to assess gene mutation, chromosome alterations, primary DNA damage, and potential for tumor induction of the subject chemical using establish protocols.

b. Perform a complete toxicological evaluation of Mononitroso-RDX when the expected human exposure is known such that a rational risk assessment can be made.

c. Provide interim protection and occupational health monitoring to personnel occupationally exposed to Mononitroso-RDX as appropriate for suspect carcinogens. Warning labels alerting personnel to the potential hazard should be posted.

HUBERT L. SNODGRASS, JR
Biologist
Toxicology Division

APPROVED:

MAURICE H. WEEKS
Chief, Toxicology Division

APPENDIX A

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4. Weisburger, J. H. and G. M. Williams, "Carcinogen Testing: Current Problems and New Approaches," Science, Vol 214, 401-407 (23 October 1981).

5. Report of Subpanel on Short Term Tests, Report of the Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation of the National Toxicology Program Board of Scientific Counselors, 17-95 (17 August 1984).

6. Lijinsky, W., "Chemical Structure and Carcinogenesis of Nitrosamines," Symposium On Structural Correlates of Carcinogenesis and Mutagenesis, Proceedings of the Second FDA Office of Science Summer Symposium, 148-157, US Naval Academy (31 August - 2 September 1977).

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8. Shimkin, M. and G. Stoner, Adv. Cancer Res., 21, 2 (1975).

APPENDIX B

ANALYTICAL QUALITY ASSURANCE

The Analytical Quality Assurance Office certifies the following:

a. These studies were conducted in accordance with:

(1) Standing Operating Procedures developed by the Toxicology Division, USAEHA.

(2) Title 21, Code of Federal Regulations (CFR), 1984 rev, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

(3) Final Rule, Pesticide Programs; Good Laboratory Practice Standards; 48 Federal Register (FR) 53963-53969, 29 November 1983.

(4) Final Rule, Toxic Substances Control; Good Laboratory Practice Standards; 48 Federal Register (FR) 53922-53944, 29 November 1983.

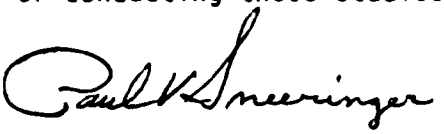
b. Facilities were inspected during its operational phase to ensure compliance with paragraph a above.

(1) 9 December 1981. Primary Eye Irritation Study with Rabbits.

(2) 11 and 14 January 1982. Primary Dermal Irritation Study With Rabbits.

(3) 22 and 24 Feb, 1 April 1982. Guinea Pig Sensitization Study.

c. The information presented in this report accurately reflects the raw data generated during the course of conducting these studies.



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